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Remaining Need for In Vitro Test to Elucidate 5-Hydroxytryptamine 2C Receptor Functioning

To the Editors:

Serotonin (5-hydroxytryptamine [5-HT]) plays an important role within the nervous system (serotonergic neurons), endocrine system (enterochromaffin cells), immune system (leucocytes), and many other organ systems.¹ The effects of serotonin are mediated by a divergent group of 5-HT receptors, including several subtypes.² Research presented in this letter involves genetic variants of the G-protein coupled 5-HT_{2C} receptor, linked to the multifunctional phosphoinositide signaling system primarily activating phosphokinase C.² 5-HT_{2C} receptors are widely distributed in the central nervous system and have been implicated in processes regulating mood, cognition, sleep, and extrapyramidal control.^{3–5} The 5-HT_{2C} receptor gene (*HTR2C*) is chromosome X-linked and contains various single nucleotide polymorphisms (SNPs), for example, rs6318, which may be functional because it results in the Cys23Ser amino acid substitution.⁶ This missense mutation has indeed been associated with dysfunction of serotonergic neurotransmission in various psychiatric disorders, and in the development of adverse effects of antipsychotic and antidepressant therapy, thus implicating functional consequences.^{7–9} Recently, Fedorenko et al¹⁰ described a possible protective effect of the 23Ser-*HTR2C* in antipsychotic drug-induced

hyperprolactinemia. However, in another patient population, an even higher association was found between hyperprolactinemia and the *G-HTR2C* allele of rs569959.¹¹ The 5-HT_{2C} receptor is an excitatory receptor displaying constitutive activity in the absence of the neurotransmitter.¹² Accordingly, full blockade of the 5-HT_{2C} receptor decreases its excitatory activity and the activation of the affected cell. This mechanism might also explain why atypical antipsychotics have fewer propensities for Parkinsonism and tardive dyskinesia than classical antipsychotic drugs.⁴ It could be argued that the 23Ser-5HT_{2C} variant will differently respond to 5-HT_{2C} antagonists, which might also have consequences for the likelihood of adverse reactions during treatment with atypical antipsychotics. However, recombinant human 23Cys-5-HT_{2C} and 23Ser-5-HT_{2C} receptors did not differ in their electrophysiological response to 5-HT when expressed in *Xenopus* oocytes.⁶ Also, experiments using COS-7 and HEK293 cell lines revealed no functional difference between these variants.¹³ Okada et al,¹⁴ however, observed higher constitutive activity of 23Ser-5-HT_{2C} than 23Cys-5-HT_{2C} receptors in Sf9 cell lines. Recombinant receptors may not adequately reflect the real life differences between genetic variants because the differences may be related to epigenetic variability or the assessed SNP may be in equilibrium with other genetic variants. Therefore, an ex vivo model to explore the functionality of genuine human 23Cys-5-HT_{2C} and 23Ser-5-HT_{2C} receptors in normal cells is urgently needed to explain the effects of the *Cys23Ser HTR2C* missense

mutation or polymorphisms, which are not in linkage disequilibrium with rs6318.

Serotonin also has an important role as immune modulator.¹ The effects may be cell-specific and depend on the expression of serotonergic components in immune cells.¹ Similarities in 5-HT function within nerve and immune cells have led to the suggestion that blood lymphocytes could be used as a convenient probe for a number of neuronal cell functions.¹⁵ Marazziti et al¹⁶ demonstrated the presence of specific messenger RNA for 5-HT_{2C} receptors in resting lymphocytes of healthy subjects and patients with obsessive-compulsive disorder and with bipolar disorder. It is thus very well possible that 5-HT_{2C} receptor's functionality can be assessed by investigating the effects of 5-HT_{2C} receptor agonists on intracellular transduction. To this end, Zhang et al¹⁷ developed and optimized a cellular inositol monophosphatase 1 assay for the characterization of 5-HT_{2C} receptor ligands using Chinese hamster ovary cells. Our aim was to use a less complicated assay to determine the functionality of 5-HT_{2C} receptors in readily available cells of human patients. We have therefore investigated whether 5-HT_{2C} induced calcium uptake by lymphocytes could be a viable alternative.

The aim of our study was to determine whether intracellular calcium could be measured upon stimulation of the 5-HT_{2C} receptor in human female peripheral mononuclear cells (PBMCs). The methods and obtained results are described in detail in the supplemental material (Supplementary Data, Supplemental Digital Content 1,

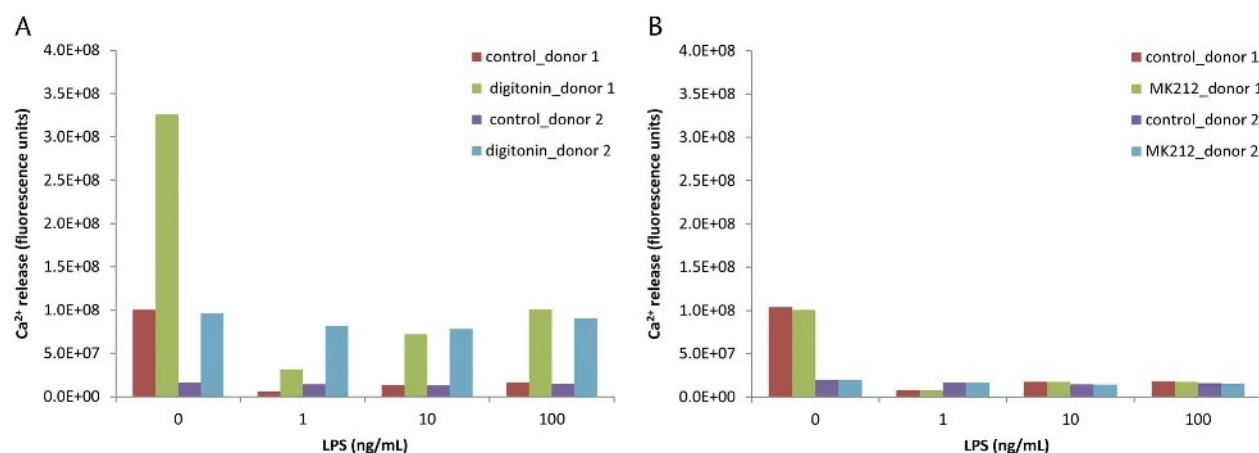


FIGURE 1. Effect of the calcium ionophore digitonin and the selective 5-HT_{2C} agonist MK212 on the calcium content of human monocytes. Freshly isolated PBMCs of 2 female donors were incubated with increasing concentrations LPS (ie, 0 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL LPS), cultured overnight, and loaded with FluoForte dye. Figure (A) represents addition of 0.2 mM digitonin and (B) addition of 1 μM MK-212.

<http://links.lww.com/JCP/A508>). In the first trial, it was measured whether application of a calcium ionophore (ie, digitonin) or direct stimulation of the 5-HT_{2C} receptor (ie, MK-212, a selective 5-HT_{2C} receptor agonist) induced intracellular calcium increases in freshly isolated PBMCs. A strong signal was obtained after addition of the calcium ionophore digitonin compared with control samples. After the addition of ATP, a clear [Ca²⁺] increase could be measured and also histamine induced a modest increase in fluorescence. However, neither α -methyl-5HT nor MK-212 induced any difference compared with control at any of the concentrations tested. In the second series of experiments, the freshly isolated PBMCs were incubated overnight (17–22 hours), with increasing concentrations of lipopolysaccharide (LPS) (ie, 0 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL). This did not induce robust differences with respect to the sensitivity to 5-HT_{2C} receptor stimulation (Fig. 1).

Hence, in human PBMCs, we failed to show any effect of 5-HT_{2C} receptor activation on intracellular free calcium levels. Also, LPS-induced monocyte activation failed to affect the sensitivity for 5-HT_{2C} receptor agonists. This failure could be due to accidental inactive 23Ser-5-HT_{2C} receptor homozygosity of the donors. However, because the *HTR2C* gene is chromosome X-linked, the frequency of the 23Ser allele in healthy Caucasian males was only 0.13⁹ and, because we used PBMCs of different female donors, this is highly unlikely.

In our opinion, it is of the utmost importance to study the activity of 5-HT_{2C} receptors in normal human *Cys23Ser HTR2C* (rs6318) carriers. It is important to note that the amino acid substitution on position 23 of the 5-HT_{2C} receptor is not necessarily causing a change in activity. The SNP can still be in equilibrium with other genetic variants, and/or the different pharmacological activity could be dependent upon the proper biochemical (epigenetic) context within human cells. Clearly, the latter can only be elucidated using suitable material of human carriers. Importantly, such a model could be an important future tool for treatment response prediction in psychiatry. Intracellular calcium release of human PBMCs may not be a viable model, so we urge our colleagues to report their serendipitous findings with other ex vivo models.

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